3.0 STEAM AUTOCLAVE TREATMENT

3.1 GENERAL DESCRIPTION OF TREATMENT TECHNOLOGY

Steam autoclave treatment combines moisture, heat, and pressure to inactivate microorganisms. This process has been used for sterilizing medical instruments in hospitals and the treatment of waste in laboratories for many years, thus the validation of autoclaving for sterilizing medical equipment and supplies is well documented. Test protocols currently exist for this purpose. Medical waste may contain many of the same pathogens as those associated with used medical instruments and supplies, however, medical waste may contain a much higher concentration of organisms in a more complex matrix. These differences make it necessary to have a unique test method specifically for the assessment of steam autoclaving as an effective medical waste treatment technology.

All steam autoclaves are constructed with a metal chamber to withstand the increased pressure/temperature required to ensure inactivation of bacteria, fungi, viruses, mycobacteria, and bacterial spores. Autoclaves come in two basic varieties, gravity displacement and prevacuum autoclaves. The size of the devices may vary from benchtop models designed to hold a single bag of waste to large commercial models which can treat more than a ton of waste per treatment cycle. The test method developed for testing the microbiological efficacy of steam autoclaving is adaptable for use in all types and sizes of autoclaves that are used for medical waste treatment.

3.1.1 Operational Parameters

The factors that affect the efficacy of steam autoclave treatment of medical waste are those affecting the internal waste load temperature, steam penetration of the waste, and the duration of treatment. These factors include:

- Temperature and pressure achieved by the autoclave
- Size of the waste load
- Composition of the waste load
- Steam penetration of the waste
- Packaging of the waste for treatment
- Orientation of the waste load within the autoclave.

3.1.1.1 Time/Temperature/Pressure/Steam Penetration

Steam autoclaves operate most effectively when the temperature measured at the center of the waste load approaches 121 °C and there is adequate steam penetration of the waste load under pressure. At a given temperature, the duration of treatment is the variable that determines the heat conduction and steam penetration to the center of a load.

3.1.1.2 Waste Characteristics and Orientation

Refer to Section 1.1 of this document for a general discussion on the description of medical waste and the specific classes that are suitable for each specific medical waste treatment technology. The waste types that are unsuitable for treatment by steam autoclaving include body parts and contaminated animal carcasses because the density of the waste items may prevent adequate treatment. Radioactive, hazardous and cytotoxic waste are also inappropriate for treatment by steam autoclaving.

3.1.1.3 Residuals

Steam autoclave treatment does not normally include a destruction step in the treatment cycle. The solid wastes remain recognizable after treatment, although they may be adequately treated to inactivate all types of microorganisms, including bacterial spores. Waste liquid from the steam condensate is usually permitted to be discharged directly to the sanitary sewer. Fugitive air emissions may escape from the treatment chamber with the steam. A grinding process can be added to the end of the treatment process which renders the waste unrecognizable and reduces it in volume.

3.1.2 Benchtop Autoclave

A benchtop autoclave is a small steel-body electric device with self-generating steam. Benchtop autoclaves are small (16 in x 17 in x 20 in) and may be only large enough to hold only a single bag of waste each treatment cycle. Prior to each use cycle, water must be added for steam generation. The controls on the autoclave are then set for the desired temperature and duration of the treatment cycle. Benchtop autoclaves are applicable for waste treatment by laboratories or clinics (dental, medical, or veterinary) that generate only small quantities of potentially infectious waste each day. If managed properly, the benchtop autoclave may serve the dual purposes of sterilizing medical supplies for use or reuse and treating potentially infectious waste.

3.1.2.1 Standard Operating Conditions

Benchtop autoclaves normally have a temperature range of 100 to 132 °C and automatic cycle timer ranges of 0 to 30 minutes or 0 to 60 minutes. Temperature and pressure gauges provide confirmation that the treatment conditions selected are achieved in the chamber. The manufacturers usually recommend this type of autoclave be operated at 121 °C and 15 psi for cycles of 15 to 60 minutes.

3.1.3 Laboratory Autoclave

Standard laboratory autoclaves vary in size from the size of the benchtop autoclave to more than twice that size (51 x 51 x 97 inches). They are similar in operation to the benchtop model except that they are equipped for direct connection to central steam lines.

3.1.3.1 Application

Standard laboratory autoclaves may be used for waste treatment by larger laboratories or clinics (dental, medical, or veterinary) that generate moderate quantities of potentially infectious medical waste each day. The laboratory autoclave device may serve the dual purposes of sterilizing medical supplies for use or reuse and treating infectious waste. If these units are used for both purposes, care should be taken to prevent cross contamination of media, instruments and equipment.

3.1.3.2 Standard Operating Conditions

Standard laboratory autoclaves have a temperature range of 100 to 132 °C. They are prepared for direct connection to steam lines with a pressure of 50 to 70 psi. The cycle timer may be set for cycles of 0 to 99 minutes.

3.1.4 Prevacuum Onsite Autoclave System

Prevacuum waste treatment autoclave systems are larger devices than the first two types of autoclaves described in this document. They are free-standing devices that may be installed outdoors. They are fully jacketed and are connected to direct steam lines. The chamber of the prevacuum autoclave is evacuated to a negative pressure prior to the addition of steam. Treatment temperatures in prevacuum autoclaves may reach ~ 135 °C and pressures reaching ~ 35 psi. Treatment cycles may vary from 30 to 55 minutes. Prevacuum autoclave waste treatment systems come in a variety of sizes and are meant for use in facilities from the size of clinics and small hospitals to large hospitals of up to 800 beds.

3.1.5 Large Volume Offsite Gravity Displacement Autoclave System

The commercial gravity displacement autoclave system is a very large device. It has an onsite steam generating boiler. This treatment system is so large it is applicable only for offsite commercial facilities. The steam is admitted to the treatment chamber through a valve in the top of the device until the pressure in the device reaches ~85 psi and the temperature exceeds 160 °C. Each cycle lasts approximately 1 hour from start to finish. Because this is an offsite treatment process it may be used by all types and sizes of generating facilities.

3.2 OPERATION EVALUATION

3.2.1 Test Organism Selection

The bacterial spores of *B. subtilis* are resistant to thermal inactivation and thus are useful as a surrogate pathogen because the organism exhibits a thermal death rate similar to the pathogenic spore-forming *Clostridium* species, which is considered one of the most heat

resistant human pathogens. The spores of the thermophilic bacterium, *B. stearothermophilus* are highly resistant to thermal inactivation and provide an added level of safety. Thus, this organism is routinely used in high concentrations to monitor the steam sterilization process of clean medical supplies.

3.2.2 Test Organism Procurement

B. subtilis (globigii) ATCC 9372 and *B. stearothermophilus* ATCC 12980 are the indicator organisms of choice for the verification of Level III and Level IV microbial inactivation respectively. Commercial suspensions may be used to prepare discs or strips with *B. stearothermophilus* or *B. subtilis*. Prepared spore strips of *B. stearothermophilus* (10^6) and *B. subtilis* (10^4) are available commercially.

3.2.3 Test Organism Quality Control

Commercially prepared spore strips should be stored according to manufacturers' directions and used before their expiration dates. If spore strips or discs are laboratory prepared, serial dilutions and platings should be made of the spore suspensions used to quantify spore concentrations prior to strip or disc inoculation.

3.2.4 Test Pack Preparation and Loading

For the test procedure, dried test spores in small envelopes containing individual spore strips or discs are placed in a thermally resistant and steam permeable container near the center of the waste load. Bacterial spores dried on paper discs and/or strips provide a significant waste treatment challenge. To establish Level IV, complete inactivation of a minimum total of 10^6 *B. stearothermophilus* spores must be consistently killed. To establish Level III microbial inactivation a minimum challenge of 10^4 *B. subtilis* spores must be completely killed.

3.2.4.1 Benchtop and Standard Laboratory Autoclave

The envelope of dried test spores should be folded and placed in a suitable container (such as a polypropylene screwcapped tube) and the lid tightened. A single hole, not to exceed 1 mm in diameter should be made in the side of the tube. For each test cycle, the packaged organisms should be placed near the center of a waste load, preferably attached to the side of the waste bag with autoclave indicator tape. If two or more bags are being treated simultaneously, the test organism package should be placed between the bags in the center of the load. The tops of the bags should be loosened or untied to allow steam to penetrate the waste load. Autoclave operators are not encouraged to place the tube of spores in a bag of waste to prevent exposure and to facilitate retrieval at the end of the autoclave cycle.

3.2.4.2 Prevacuum Onsite Autoclave System

The indicator challenge is prepared by placing spore strips or discs containing *B. subtilis* (10^4) and/or *B. stearothermophilus* (10^6) into a suitable 4 oz screw-capped container such as Nalgene Teflon PFA. Teflon PFA has a high thermal resistance and a non-sticking property. Four holes should be drilled in the top and bottom of the container to provide access for the steam. The container should be suspended from the top of the autoclave chamber, with medical waste bags packed against and around it. The tops of the waste bags need not be loosened or untied if holes are made in the bags.

3.2.4.3 Large Volume Offsite Gravity Displacement Autoclave System

The test challenge is prepared and packaged as described for the prevacuum autoclave system. The test container is suspended in the bottom one-third of the waste bin by a metal chain that is attached with a metal bolt to the bottom of the test pack container. The chain is suspended from a metal pipe (one inch or greater diameter) that is placed across the waste bin. The remainder of the standard waste load is then packed around and on top of the microbial challenge.

3.2.5 Test Pack Exposure and Removal

3.2.5.1 Benchtop and Standard Laboratory Autoclave

The autoclave is operated under normal conditions. At the conclusion of the cycle the test organism envelopes are removed from the load, allowed to air dry, and processed within 24 hours according to the recovery procedure. It is important to keep the treated indicator spores from temperature extremes.

3.2.5.2 Prevacuum Onsite Autoclave System

After completion of the normal prevacuum autoclave treatment cycle and before waste compaction, the test organism container is removed from the autoclave, test organism envelopes are allowed to air dry, and processed within 24 hours according to the recommended recovery procedure.

3.2.5.3 Large Volume Offsite Gravity Displacement Autoclave System

The autoclave is run at normal operating conditions. Within five to ten minutes of the waste leaving the autoclave, the test container should be removed from the receptacle either manually or with the aid of a fork lift. The test organism envelopes are removed, allowed to air dry, and then processed within 24 hours according to the recommended recovery procedure.

3.2.6 Organism Recovery

To recover test organisms, test discs or strips should be inoculated aseptically into 5.0 mL soybean-casein digest broth medium (or equivalent) and incubated for at least 72 hours followed by observation for visible growth. Additional spore discs or strips are processed as viability, media, and incubation controls. Samples inoculated with *B. stearothermophilus* are incubated at 55 °C, while those samples inoculated with *B. subtilis* are incubated at 32 °C.

At the end of the required incubation time, media that were inoculated with the test organisms are examined for turbidity as an indication of microbial growth. If growth is noted it is a preliminary sign that some indicator spores survived the treatment process. To confirm the identity of the organisms present in the media demonstrating growth, the positive test cultures are subcultured onto soybean-casein digest agar plates (or equivalent) and incubated at the appropriate temperature (55 °C or 32 °C) for at least 24 hours. The plates are then examined and compared to the growth of subcultured growth controls to confirm the identity of the organisms present in the sample as the indicator organisms. Level III microbial inactivation is indicated by the inactivation of a minimum of 10^4 *B. subtilis* spores. If Level IV microbial inactivation is desired, a minimum of 10^6 *B. stearothermophilus* must be completely killed.

3.2.7 Treatment Validation and Testing Frequency

To validate the treatment process, duplicate cycles should be tested on each of three different days. If results show surviving spores, then the treatment process parameters (time, temperature, pressure) should be checked and/or modified and the validation testing repeated until results are satisfactory. Once the appropriate operating parameters are established that ensure adequate waste treatment, the process should be monitored bi-weekly as suggested in Table 1.2 in Section 1.3.7. Temperature charts and pressure gauges may be used to monitor daily processing for indications of upset conditions. Upset conditions should be recorded and maintenance performed. Testing should then be repeated.

3.2.8 Quality Control Procedures

Quality control procedures presented in Section 1.3.7 should be followed.